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ORIGINAL ARTICLE

The teratogenic effects of sediments from the Yangtze Estuary and adjacent bay, China, on frog embryos

Huahong Shi · Jing Yuan · Zhijun Dai · Hongyi Yao

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Abstract In recent decades, the Yangtze (Changjiang) Estuary has suffered great environmental changes due to intensive human activities in the Yangtze River basin. This study assessed the ecotoxicity of sediments from the Yangtze Estuary with the frog embryo teratogenesis assay—*Xenopus* (FETAX). The results showed that the sediment extracts induced multiple malformations in embryos of *Xenopus tropicalis*. In the embryos treated with nearshore extracts, abnormal eyes, narrow fins and hypopigmentation were the dominant phenotypes, followed by enlarged proctodeums. In these embryos, the percentages of total malformations were greater than 50 % at five sampling sites and less than 40 % at the other seven sites. However, in the embryos treated with the offshore extracts, elongation of the proctodeums was the dominant phenotype, followed by bent tails. The percentages of total malformations ranged from 25 to 45 %. Comparatively, the embryos treated with extracts from the nearshore zone showed a greater variety of phenotypes than those treated with extracts from the offshore zone. These results suggest that the sediments showed high teratogenicity to the amphibian embryos. The results also indicate that the teratogencity to *X. tropicalis* embryos is a useful indicator of the pollution of sediments.

H. Shi (✉) · Z. Dai (✉) · H. Yao
State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai 200062, China
e-mail: hhshi@des.ecnu.edu.cn

Z. Dai
e-mail: zjdai@sklec.ecnu.edu.cn

H. Shi · J. Yuan
Department of Environmental Science, Key Laboratory of Urbanization and Ecological Restoration, East China Normal University, Shanghai 200062, China

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Introduction

The Yangtze (Changjiang) Estuary is one of the largest world's estuaries. The estuarine region has a population of more than 15 million and provides great ecosystem services for wildlife and humans. For example, the Yangtze Estuary is the most important drinking water source for Shanghai, China. With the development of industry and agriculture in the Yangtze River basin, substantial quantities of pollutants have been produced. These pollutants have spread rapidly to the Yangtze River and have ultimately been transported as solid aggregates or dispersed in the water to the estuary (Dai et al. 2011a). Moreover, polluted water resulting from industry, agriculture and resource consumption by the local population has increased markedly along the shoreline of the estuary. These anthropogenic activities led to substantial changes in the estuarine environment (Salazar-Coria et al. 2010).

Many investigations have been performed on the environment of the Yangtze Estuary. The topics examined by these investigations include salt intrusion, red tide blooms and hypoxia in the bottom water (Dai et al. 2011a, b; Gao et al. 2011). In addition, a few investigations have examined the levels of heavy metals and organic pollutants in the Yangtze Estuary (An et al. 2009; Deng et al. 2010; Liu et al. 2008). In addition to many typical pollutants, several emerging contaminants, such as perfluorooctane sulfonate and pharmaceuticals, are also found in the Yangtze Estuary (Pan and You 2010; Yang et al. 2011).

Estuarine sediments from a river system can serve as repositories of information in the field. The information that they furnish includes not only for ambient bottom water environmental conditions, but also for the status of pollutants in the system. Sediments can bind pollutants as well as releasing them (Pan and You 2010). Consequently, sediment contamination has substantial effects on water quality and the ecological status of water bodies (Yan et al. 2011). The Yangtze Estuary is particularly characterized by high sediment concentrations and strong dynamic conditions (Dai et al. 2012; Liu et al. 2010). Contaminated suspended sediments are highly toxic to aquatic organisms (Hill et al. 2009). However, few data are available on the ecotoxicological effects of the Yangtze Estuary sediments on aquatic organisms, and information on the health risk to humans posed by these sediments is even scarcer.

Acute bioassays for estuarine sediments have been designed for algae, amphipods, copepods, sea urchins and fishes (Bellas et al. 2011; Moreno-Garrido et al. 2007). The frog embryo teratogenesis assay—*Xenopus* (FETAX) is a high-throughput toxicological test used to assess ecological and human health hazards (American Society for Testing and Materials (A.S.T.M.) 1998). *Xenopus tropicalis* is an emerging animal model in developmental biology and ecotoxicology (Berg et al. 2009). It is closely related to *X. laevis* and offers the advantages of a smaller size and shorter life cycle compared with this congener (Hirsch et al. 2002). A particular advantage of this assay is that the results of FETAX can be validly extrapolated to human health due to the close relationship between amphibians and humans.

In this paper, embryos of *X. tropicalis* were exposed to sediment extracts from the Yangtze Estuary and the adjacent bay. The purpose of this paper was to assess the developmental toxicity of sediment extracts from the Yangtze Estuary and the adjacent bay.

Materials and methods

Study area and sample collection

The Yangtze Estuary is a typical braided-type estuary. The morphodynamic patterns present from Xuliujing to the mouth of the estuary can be described as "three-order bifurcations and four-outlet diversions into the sea". The estuary is a meso-tidal estuary with average tides ranging from 2.4 to 3.2 m. In the estuarine area, the water depth on the tidal flat changes frequently in time and space to create a unique hydrodynamic environment on the tidal flat. This environment controls the processes of sediment transport. Twenty-four sampling sites were defined in two regions, termed nearshore (N) and offshore (O), in the estuarine area and the adjacent bay (Fig. 1). The sediment samples were collected with a grab (clamshell) sampler during May 2011. All samples were shipped to the laboratory and stored at 4 °C.

Preparation of sediment extracts

The aqueous sediment extracts were prepared following the methods of Fort et al. (2001), with some modifications. Briefly, a quantity of FETAX solution at 4:1 dilution (v/w) was mixed with the sediment sample in a 500 ml brown

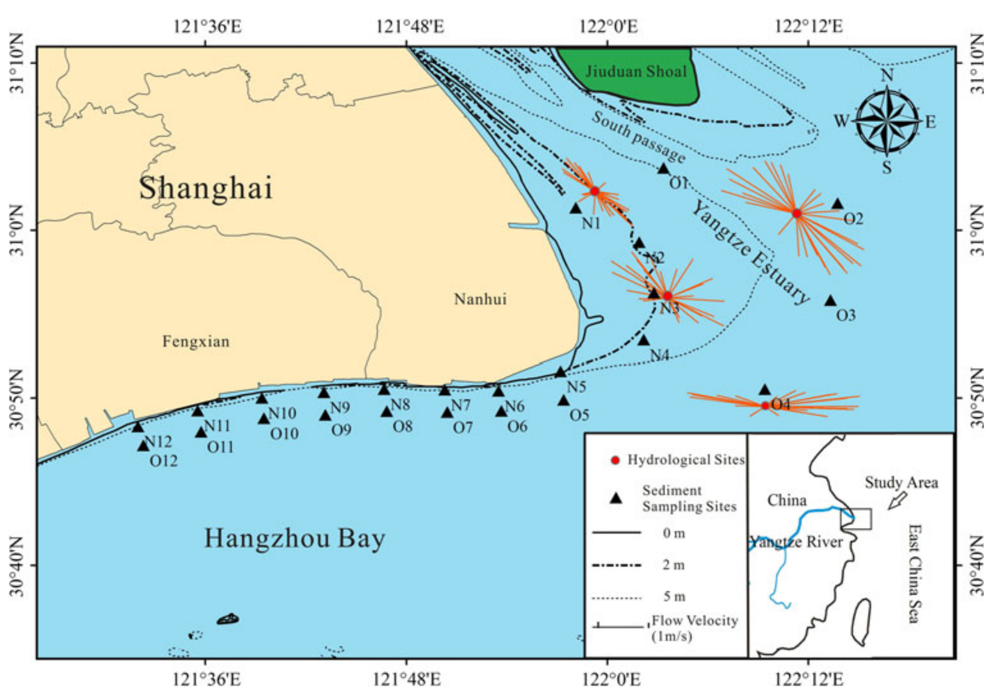

Fig. 1 Sampling sites for sediments from the Yangtze (Changjiang) Estuary and the adjacent bay, China

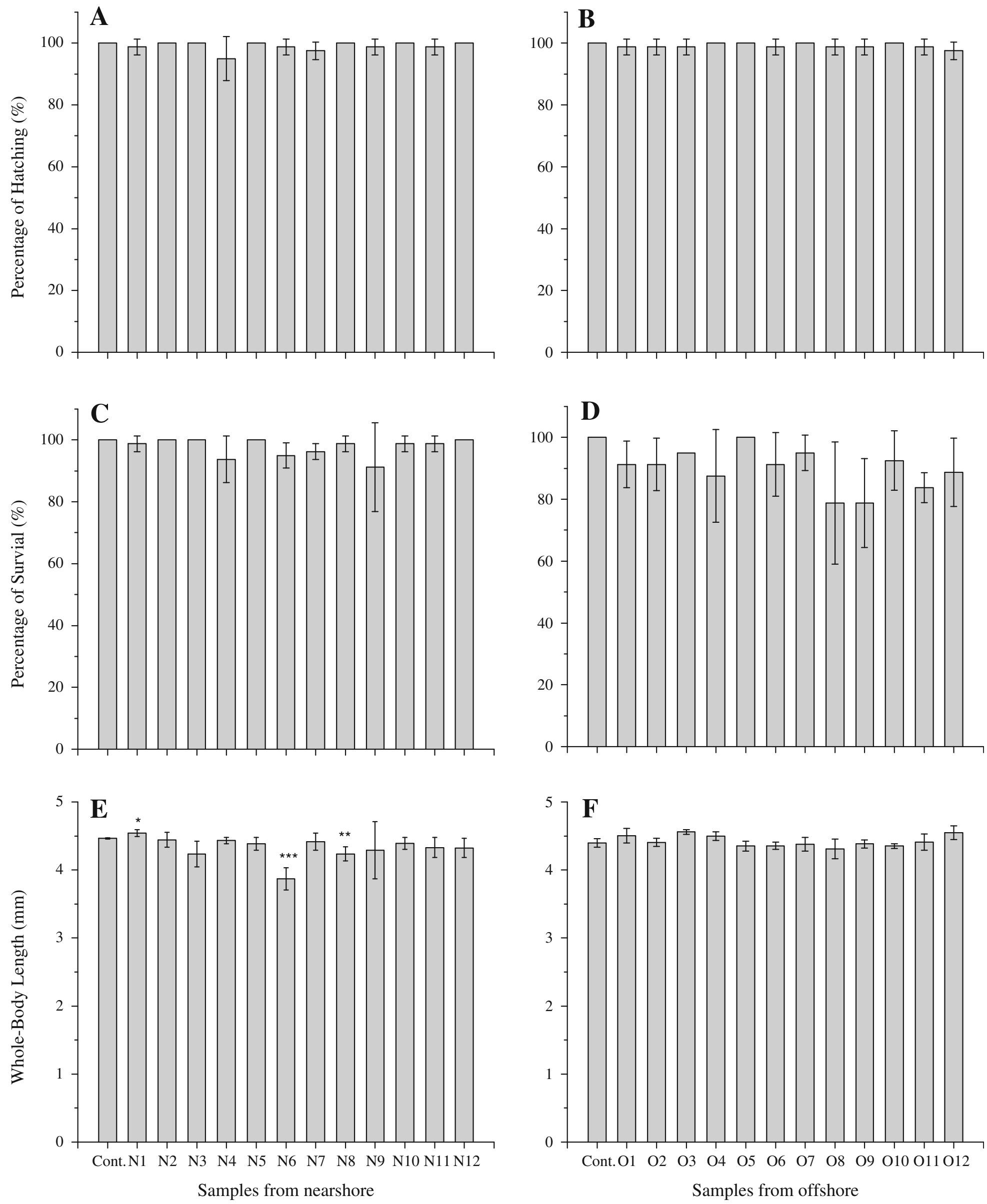

Fig. 2 Effects of sediment extracts on survival and growth of *Xenopus tropicalis* embryos after 48 h exposure ($n = 4$). A one-way analysis of variance was used with $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ for comparisons of the treatment groups with the control group

glass bottle. Specifically, 400 ml of FETAX solution was mixed with 100 g of sediment to minimize headspace and to reduce volatilization. The aqueous soil mixtures were then tumbled in a rotary extractor for 48 h at 30 ± 2 rpm and 22 ± 2 °C in the dark. The tumbled samples were allowed to settle overnight at 4 °C. The samples were then centrifuged for approximately 20 min at 8,000 rpm until the supernatant was completely clear. The extract was decanted, and the pH and dissolved oxygen were measured. None of the samples deviated from the acceptable pH range of 6.5–9.0. The extracts were stored at 4 °C prior to toxicological testing.

Exposure experiments

The use of live organisms in this study followed the protocols approved by the Science and Technology Commission of Shanghai Municipality. These protocols ensure that the experimental procedures adhere to national guidelines for the protection of human subjects and animal welfare. The husbandry of *X. tropicalis* adults and the breeding of the experimental animals were performed as described previously (Guo et al. 2010). Six pairs of adult frogs were used for breeding, and healthy embryos from three pairs of frogs were collected and combined for the experiments.

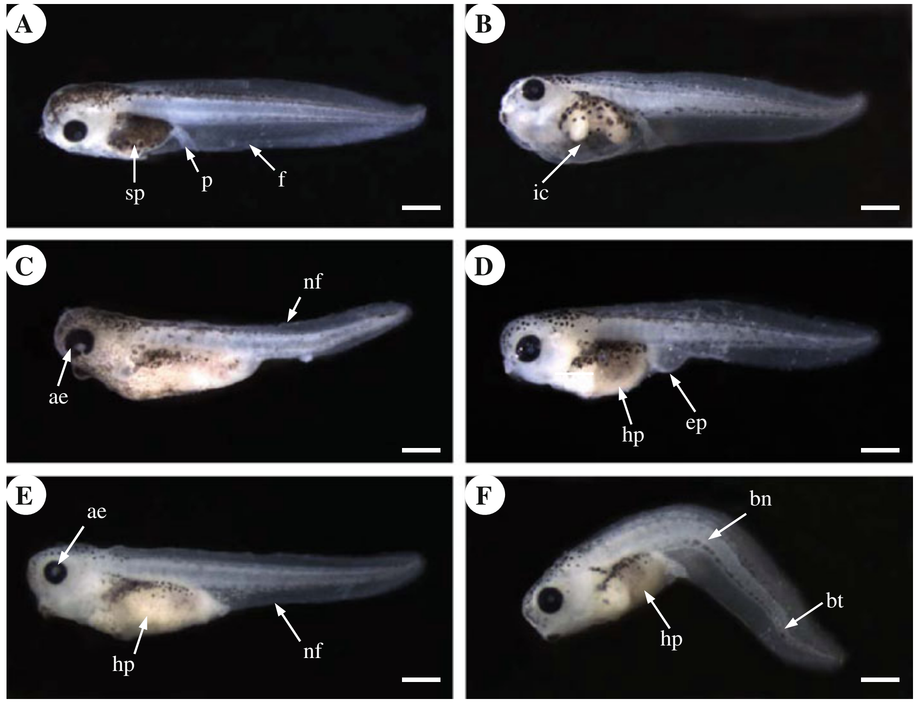

Fig. 3 Morphological photographs of *Xenopus tropicalis* embryos in the control (**a**) and treatment (**b–f**) groups after 48 h exposure. *ae* abnormal eye, *bn* bent notochord, *bt* bent tail, *ep* enlarged proctodeum, *f* fin, *ic* intestinal coiling, *nf* narrow fin, *p* proctodeum, *hp* hypopigmentation, *sp* skin pigmentation. *Scale bar* 0.5 mm

The exposure experiments were conducted following FETAX, with some modifications (American Society for Testing and Materials (A.S.T.M.) 1998). A total of 5 ml of sediment extract was put into each well of a plate with 24-well plates. Embryos at the same stage were chosen for the exposure experiments. Ten embryos were placed in each well. Four replicates were used in each group. The plates were incubated at 26 ± 0.5 °C for 24 h under dark to avoid the photodecomposition of organic pollutants. The dead embryos were removed from the dish, and the medium was renewed at 24 h intervals.

Observations and measurements of embryos

After 48 h of exposure, the surviving embryos were collected from all the groups and anesthetized with 100 mg/L MS-222. The embryos were then fixed with 4 % formalin for 24 h, washed with tap water, and preserved in 70 % ethanol. The embryos were observed under an Olympus SZX16 dissecting microscope (Olympus, Tokyo, Japan), and images were taken with an Olympus DP 25 camera. The whole-body length was measured in each replicate dish with computer-assisted image analysis (iSee V3.873). The malformations observed were distinguished from the changes due to the delayed development of the embryos, and the main phenotypes represented by the observed malformations were determined. The calculation of the percentage of teratogenicity was based only on the number of surviving embryos.

Data analysis

The data were analyzed with SPSS16.0 software. Each well of 10 embryos was considered as a replicate, and each group included four replicates ($n = 4$). The differences among the mean values for the control and the treatments were determined by a one-way analysis of variance (ANOVA) followed by a two-sided Dunnett test.

Results and discussion

Effects on survival and growth

There was no significant difference on the percentages of hatching and survival between the control and the treatment groups (Fig. 2a–d). The whole-body length increased by 1.8 % ($p < 0.05$) in N1 and decreased by 13.3 % ($p < 0.001$) in N6 and by 5.1 % ($p < 0.01$) in N8 (Fig. 2e). These results suggest that the sediment extracts from the study sites in the Yangtze Estuary had no significant effects on hatching and survival and slight effects on the growth of the amphibian embryos. The inhibition of growth is also used in FETAX as an index to indicate the effects of test chemicals (American Society for Testing and Materials (A.S.T.M.) 1998). Compared with single compounds, environmental samples have a more complex composition. Some substance providing nutrition to the developing embryos might be present in the sediment extracts. Certain

Table 1 Percentage of principal phenotypes of malformations (%) at nearshore (N) and offshore (O) sampling sites

Sites	Abnormal eyes	Elongation proctodeum	Enlarged proctodeum	Narrow fin	Bent tail	Hypopigmentation
Nearshore sites						
Cont.	0 ± 0[a]	0 ± 0	5.0 ± 0	1.3 ± 2.5	0 ± 0	0 ± 0
N1	2.6 ± 3.0	1.3 ± 2.5	6.3 ± 4.8	5.0 ± 4.1	5.0 ± 4.1	11.4 ± 4.7**[b]
N2	8.8 ± 2.5***	1.3 ± 2.5	3.8 ± 2.5	1.3 ± 2.5	0 ± 0	5.0 ± 4.1
N3	12.5 ± 2.9***	5.0 ± 4.1	23.8 ± 24.3	31.3 ± 4.8***	3.8 ± 4.8	71.3 ± 32.5**
N4	10.5 ± 3.8**	0 ± 0	5.5 ± 4.6	6.7 ± 5.0	4.2 ± 5.6	13.3 ± 7.0**
N5	6.3 ± 2.5**	2.5 ± 5.0	11.3 ± 8.5	18.8 ± 6.3**	1.3 ± 2.5	16.3 ± 6.3**
N6	57.8 ± 13.0***	0 ± 0	70.7 ± 25.1**	96.1 ± 7.9***	11.8 ± 6.6*	96.1 ± 7.9***
N7	20.9 ± 6.3**	0 ± 0	11.6 ± 4.8*	15.5 ± 6.8**	2.6 ± 3.0	14.3 ± 2.5***
N8	16.5 ± 5.1**	0 ± 0	20.4 ± 7.8**	53.4 ± 11.8***	10.2 ± 4.4**	28.0 ± 7.2***
N9	10.5 ± 7.7*	0 ± 0	33.4 ± 40.1	52.4 ± 27.3*	12.5 ± 25	28.3 ± 43.3
N10	5.1 ± 4.1*	2.5 ± 2.9	19.1 ± 9.8*	32.9 ± 8.5***	3.8 ± 7.5	16.5 ± 7.7**
N11	6.4 ± 4.9*	0 ± 0	17.8 ± 9.7*	29.0 ± 11.6**	2.5 ± 2.9	19.0 ± 10.4*
N12	16.3 ± 4.8***	1.3 ± 2.5	33.8 ± 13.1**	41.3 ± 17.0**	11.3 ± 11.1	65.0 ± 28.0**
Offshore sites						
Cont.	0 ± 0	0 ± 0	1.3 ± 2.5	2.5 ± 2.9	1.3 ± 2.5	2.5 ± 2.9
O1	5.4 ± 6.3	33.0 ± 6.5***	4.2 ± 5.6	4.2 ± 5.6	12.8 ± 9.8	5.4 ± 4.3
O2	6.8 ± 2.2**	39.2 ± 8.6***	1.6 ± 3.1	7.2 ± 5.7	11.2 ± 5.5*	5.6 ± 4.3
O3	1.3 ± 2.6	27.6 ± 15.1*	0 ± 0	10.5 ± 4.3*	13.2 ± 12.5	2.6 ± 3.0
O4	4.9 ± 6.1	32.1 ± 6.7***	0 ± 0	15.1 ± 7.7*	13.5 ± 6.8*	12.2 ± 6.9*
O5	2.5 ± 2.9	18.8 ± 8.5**	0 ± 0	6.3 ± 7.5	6.3 ± 6.3	17.5 ± 9.6*
O6	12.4 ± 8.6*	29.9 ± 7.1***	1.5 ± 2.9	9.6 ± 2.7*	11.0 ± 6.3*	13.0 ± 12.2
O7	5.3 ± 4.5	30.0 ± 7.4***	1.3 ± 2.5	5.3 ± 4.5	4.0 ± 2.7	10.4 ± 3.9*
O8	16.6 ± 13.7	22.9 ± 14.7*	0 ± 0	6.2 ± 4.7	12.9 ± 5.7*	4.4 ± 5.3
O9	7.0 ± 7.0	26.6 ± 3.3***	4.2 ± 8.3	1.3 ± 2.6	25.8 ± 19.1*	10.2 ± 5.4*
O10	8.0 ± 2.4**	26.5 ± 9.4**	1.3 ± 2.5	3.8 ± 4.8	9.3 ± 8.4	5.1 ± 4.1
O11	13.4 ± 5.7**	11.7 ± 7.6*	3.1 ± 6.3	4.7 ± 9.4	16.7 ± 8.1*	7.5 ± 5.9
O12	0 ± 0	22.9 ± 9.2**	0 ± 0	0 ± 0	5.5 ± 4.4	2.9 ± 3.4

[a] Each value represents the mean ± SD of four replicates ($n = 4$). The values shown are the percentages of the surviving embryos showing the specified malformations

[b] A one-way ANOVA followed by a two-sided Dunnett test was used with * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ for comparisons of the treatment groups with the control group

contaminants also have hormesis effects at low concentrations (Murado and Vazquez 2007). Therefore, the inhibition of growth might not represent an ideal index for assessing the developmental toxicity of environmental samples in FETAX.

Different phenotypes represented in the malformations

Multiple malformations were observed in the groups treated with sediment extracts. The main phenotypes represented in the malformations included abnormal eyes, abnormal intestinal coiling, narrow fins, a bent notochord, an enlarged proctodeum, and posterior anus and skin hypopigmentation (Fig. 3). The phenotypes varied not only between nearshore and offshore, but also among sites (Table 1). In the embryos treated with nearshore extracts, abnormal eyes, narrow fins and hypopigmentation were the dominant phenotypes, followed by enlarged proctodeums. The percentage of narrow fins reached 96 % in N6. However, in the embryos treated with the offshore extracts, elongation of the proctodeums was the dominant phenotype, followed by bent tails. The percentage of any single phenotype did not exceed 40 % in the groups treated with offshore extracts (Table 1).

In the groups treated with nearshore extracts, the percentages of total malformations were greater than 50 % at five study sites and less than 40 % at the other seven sites (Fig. 4). All the embryos showed malformations in N6. The percentages of total malformations ranged from 25 to 45 % in the groups treated with offshore extracts. The

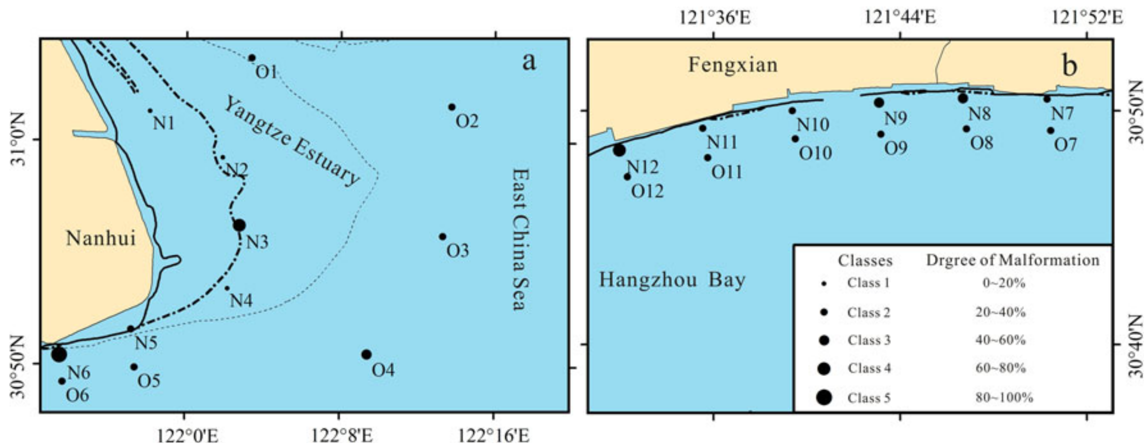

Fig. 4 Degree of malformation of *Xenopus tropicalis* embryos induced by sediment extracts from the Yangtze (Changjiang) Estuary. The size of the symbol (*filled circles*) indicates the classes of the total percentage of malformations

embryos treated with the extracts from the nearshore zone showed a greater variety of phenotypes than the embryos treated with the extracts from the offshore zone (Table 1).

The frog embryo teratogenesis assay—*Xenopus* (FETAX) is a useful method for evaluating the developmental toxicity of chemicals. The main endpoints specified in FETAX include lethality, total teratogenicity, and growth inhibition (American Society for Testing and Materials (A.S.T.M.) 1998). However, the specific phenotypes represented by the malformations receive little attention (Baba et al. 2009). The phenotypes observed among the malformations in the embryos can provide clues that are useful for identifying the mechanisms underlying the teratogenicity induced by chemicals. Phenotype-based assays with *Xenopus* embryos have been used widely in genetic screening (Wheeler and Brändli 2009). The specific phenotypes observed might be used as endpoints to facilitate additional uses beyond acute toxicity testing in FETAX (American Society for Testing and Materials (A.S.T.M.) 1998).

Teratogenicity to embryos used as an indicator of pollution

In previous studies, FETAX has been successfully used to assess the toxicity of sediment samples (Fort et al. 2001). In this study, the degree of teratogenicity and the phenotypes observed among the malformations indicated differences in the status of the pollution of sediments from the Yangtze Estuary. Conversely, the occurrence of pollution in specific areas is affected by various dynamic processes, including tides, discharge, waves, wind and flocculation (Dai et al. 2012). The local dynamic conditions produce divergent sediment characteristics, including the pollution status of the sediments (Liu et al. 2010).

The pollutants in the nearshore area originate from diverse sources, including local industry, the consumption of resources by the local population, and shipyards. In contrast, the pollutants in the offshore area originate primarily from the upstream reaches of the Yangtze River (Dai et al. 2011a; Deng et al. 2010). This difference might be the reason that a greater variety of malformations was produced by the sediments from the nearshore area than those from the offshore area. In particular, relatively high teratogenicity was produced by the sediments from sites N6 and N12, which are located near two large-scale chemical industry facilities on the shore. Site N3 is located in the area of maximum turbidity in the estuary and is exposed to the combined effects of runoff and tidal currents. It is probable that suspended matter, including matter that has adsorbed pollutants, will settle to the bottom in an area with these special characteristics. Therefore, the teratogenicity associated with the sediments from N3 was relatively high.

Conclusions

In brief, the sediment extracts from the Yangtze Estuary induced multiple malformations in embryos, and the phenotypes represented by these malformations varied not only between the nearshore and offshore areas but also among the sampling sites. These results suggest that the sediments from the Yangtze Estuary and the adjacent bay showed high teratogenicity to amphibian embryos. The results also indicate that teratogenicity to *X. tropicalis* embryos is a useful indicator of pollution in sediments.

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